

$$A \leftarrow \begin{array}{l} \text{(Fuc)}_{i} \\ \text{(GlcNAc-(Gal))}_{i} \\ \text{(GlcNAc-GlcNAc-Man)} \\ \text{(GlcNAc-GlcNAc-Man)} \\ \text{(IGlcNAc-(Gal))}_{i} \\ \text{(Sia)}_{i} \\ \text{(Sia)}_{i} \\ \text{(R)}_{w} \\ \text{(Sia)}_{i} \\ \text{(Sia)}_{i} \\ \text{(R)}_{w} \\ \text{(Sia)}_{i} \\ \text{(Sia)}_{m} \\ \text{(R)}_{y} \\ \text{(R)}_{w} \\ \text{($$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 39A

```
CHO, BHK, 293 cells, Vero expressed Cerezyme a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 39B

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- Sialidase
 CMP-SA-M-6-P (1.2 mol eq),
- ST3Gal3
 3. CMP-SA (16 mol eq), ST3Gal3
- 5. CM -5A (10 mol eq), 515Gai.

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = mannose-6-phosphate

FIG. 39C

```
CHO, BHK, 293 cells, Vero expressed Cerezyme.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol ec).
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = Mannose-6-phosphate
```

ST3Gal3
3. CMP-SA, ST3Gal3

FIG. 39D

```
CHO, BHK; 293 cells, Vero expressed Cerezyme.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

```
1. CMP-SA-levulinate, ST3Gal3,
buffer, salt
2. H<sub>4</sub>N<sub>2</sub>-spacer-M-6-P or clustered M-6-P
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = M-6-P or clustered M-6-P
```

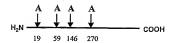
FIG. 39F

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 39F



$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc\text{-}(Gal)}_{a}]_{a^{-}} & (\mathrm{Sia})_{j}^{-} & (\mathrm{R})_{v} \\ [\mathrm{GlcNAc\text{-}(Gal)}_{a}]_{a^{-}} & (\mathrm{Sia})_{j}^{-} & (\mathrm{R})_{v} \end{bmatrix} \\ [\mathrm{GlcNAc\text{-}(Gal)}_{a}]_{a^{-}} & (\mathrm{Sia})_{a^{-}} & (\mathrm{R})_{w} \end{bmatrix}_{q} \\ [\mathrm{GlcNAc\text{-}(Gal)}_{a}]_{a^{-}} & (\mathrm{Sia})_{m^{-}} & (\mathrm{R})_{y} \end{bmatrix}_{q} \\ [\mathrm{GlcNAc\text{-}(Gal)}_{a}]_{b^{-}} & (\mathrm{GlcNAc\text{-}(Gal)}_{a})_{b^{-}} & (\mathrm{GlcNAc\text{-}(Gal)}_{a})_{b^{-}} & (\mathrm{GlcNAc\text{-}(Gal)}_{a})_{b^{-}$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 39G

```
\label{eq:linear_constraints} \begin{split} & \text{Insect cell expressed Cerezyme.} \\ & \text{a-d, } f, h, j\text{-m, } s, u, v\text{-y} = 0; \\ & \text{e, } g, i, q, r, \text{ t (independently selected)} = 0 \text{ or } 1. \end{split}
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 39H

```
Yeast expressed Cerezyme.

a-m=0; q-y (independently selected) = 0 to 1;

p=1; R (branched or linear) = Man, oligomannose.
```

```
1. Endoglycanase
2. Galactosyltransferase, UDP-Gal
▼ 3. CMP-SA-PEG, ST3Gal3
```

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

FIG. 391

PCT/US2004/011494

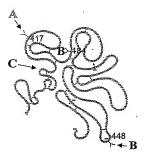
155/497

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-transferrin.

FIG. 39J



$$B \leftarrow \begin{array}{l} \text{(Fuc)}_{i} \\ B \leftarrow \begin{array}{l} \text{GlcNAc-Gal)}_{s}_{l}_{c} \cdot (\text{Sa)}_{j} \cdot (\text{R})_{v} \end{array} \Big]_{r} \\ \text{(R')}_{o} \end{array} \\ \text{Man} \left[\begin{array}{l} \text{[GlcNAc-(Gal)}_{s}]_{e}^{-} \cdot (\text{Sia})_{j} \cdot (\text{R})_{v} \end{array} \right]_{r} \\ \text{[GlcNAc-(Gal)}_{e}]_{g} \cdot (\text{Sia})_{i} \cdot (\text{R})_{x} \end{array} \Big]_{r} \\ \text{Man} \left[\begin{array}{l} \text{[GlcNAc-(Gal)}_{d}]_{h}^{-} \cdot (\text{Sia})_{m}^{-} \cdot (\text{R})_{x} \end{array} \right]_{r} \\ \text{[GlcNAc-(Gal)}_{d}]_{h}^{-} \cdot (\text{Sia})_{m}^{-} \cdot (\text{R})_{y} \end{array} \right]_{r} \\ \text{[GlcNAc-(Gal)}_{d}]_{h}^{-} \cdot (\text{Sia})_{m}^{-} \cdot (\text{R})_{y} \end{array} \Big]_{r} \\ \text{(R')}_{o} \left[\begin{array}{l} \text{Result of } (\text{Result of } \text{Result of }$$

$$C \longleftarrow \text{-(Fuc)}_{0\text{-}1} \qquad A \longleftarrow \text{-GlcNAc-GlcNAc-Man} \qquad \begin{array}{c} \text{Man-[Man]}_{0\text{-}12} \\ \text{Man} \end{array} \begin{array}{c} \text{[Man]}_{0\text{-}6} \\ \text{[Man]}_{0\text{-}6} \end{array}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 40A

WO 2004/099231 PCT/US2004/011494

157/497

```
CHO, BHK, 293 cells, Vero expressed tPA
a-g, n = 1; h = 1 to 3;
j-m, i, (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1; o, v-y = 0.
```

```
1. Mannosidase(s), sialidase
2. GNT1,2 (4 and/or 5) UDP-GlcNAc
3. Gal transferase, UDP-Gal

4. CMP-SA-PEG, ST3Gal3
```

```
A = B; a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1;
o = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG
```

FIG. 40B

```
Insect or fungi cell expressed tPA A=B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1&2, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal

▼ 3. CMP-SA-PEG. ST3Gal3
```

```
\begin{array}{lll} A=B; & b,d, \ f, \ h, \ k, m,o,s,u,w,y=0;\\ a,c,e,g,i,r,t \ (independently \ selected)=0 \ or \ 1;\\ n=1; \ j,l,v,x \ (independently \ selected)=0 \ or \ 1;\\ R=PEG. \end{array}
```

FIG. 40C

Yeast expressed tPA B = A; i = 0.

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

FIG. 40D

Insect or fungi cell expressed tPA
A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. alpha and beta mannosidases
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

FIG. 40E

```
Insect or fungi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1&2, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

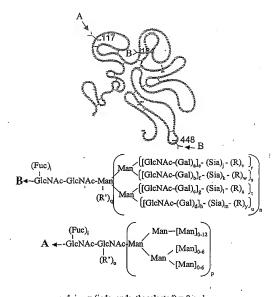
FIG. 40F

```
Insect or fingi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.
```

- GNT's 1 & 2, UDP-GlcNAc
 Galactosidase (synthetic enzyme)
- Galactosidase (synthetic enzyme), PEG-Gal-F.

```
A=B;\ b,d,\ f,\ h,\ j\text{-o},\ s,\ u,\ w,\ y=0; a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1; n=1; R=PEG.
```

FIG. 40G



a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 40H

```
NSO expressed tPA
A = B; a-m, r-u (independently selected) = 0 or 1;
n = 1; o, p, q, v-v = 0
```

```
1. sialidase, alpha-galactosidase
2. CMP-SA-levulinate, ST3Gal3,
```

```
A = B; a-m, r-y (independently selected) = 0 or 1;
n = 1; o, p, q = 0;
v-v (independently selected) = 1,
 when j-m (independently selected) is 1;
R = PEG.
```

FIG. 401

```
CHO, BHK, 293 cells, Vero expressed tPA
a-g, n, p = 1; h = 1 \text{ to } 3;
j-m, i, (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1; q, o, v-y=0.
```

- alpha and beta Mannosidases
 CMP-SA, ST3Gal3
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1; o = 1;
q, p, v-y=0; j-m (independently selected) = 0 or 1;
R' = Gal-PEG
```

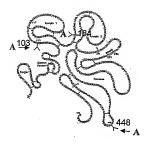
FIG. 40.1

Plant expressed tPA A = B; a-d, f, h, j- m, s, u, v-y=0; e, g, i, q, r, t (independently selected) = 0 or 1; n=1; $R^2 = xylose$

- 1. hexosaminidase,
- alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

A = B; a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0; q = 1; R' = GlcNAc-PEG.

FIG. 40K



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & \\ -(\operatorname{GlcNAc-Glc})_{s}l_{r} & (\operatorname{Sia})_{s}^{-} & (\operatorname{Sia})_{s}^{-} & (\operatorname{R})_{w} \\ -(\operatorname{GlcNAc-Glc})_{s}l_{r}^{-} & (\operatorname{Sia})_{s}^{-} & (\operatorname{R})_{w} \end{bmatrix}_{s} \\ + (\operatorname{GlcNAc-Glc})_{s}l_{r}^{-} & (\operatorname{Sia})_{s}^{-} & (\operatorname{R})_{w} \end{bmatrix}_{s} \\ + (\operatorname{GlcNAc-Glc})_{s}l_{r}^{-} & (\operatorname{Sia})_{s}^{-} & (\operatorname{R})_{w} \end{bmatrix}_{s} \\ + (\operatorname{GlcNAc-Glc})_{s}l_{r}^{-} & (\operatorname{Sia})_{r}^{-} & (\operatorname{R})_{y} \end{bmatrix}_{u} \\ + (\operatorname{GlcNAc-Glc})_{s}l_{r}^{-} & (\operatorname{Sia})_{r}^{-} & (\operatorname{R})_{y} \end{bmatrix}_{u}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. $^{\prime}$ v-y = 0; R = polymer.

FIG. 40L

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 40M

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (1.2 mol eq),
ST3Gal3
    CMP-SA (16 mol eq), ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 40N

NSO expressed TNK tPA
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.

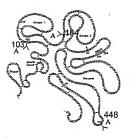
- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 400

WO 2004/099231 PCT/US2004/011494

166/497



$$\mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{a}]_{a}^{-}(\mathrm{Sia})_{j}^{-}(R)_{v} \\]_{t} \\ \mathrm{GlcNAc\text{-}}(\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{b}]_{t}^{-}(\mathrm{Sia})_{k}^{-}(R)_{w} \end{bmatrix}_{t}^{-} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{b}]_{t}^{-}(\mathrm{Sia})_{k}^{-}(R)_{w} \\]_{t} \\ \mathrm{Man} \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{a}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \end{bmatrix}_{t} \\ [[\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{m}^{-}(R)_{y} \end{bmatrix}_{u}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{m}^{-}(R)_{y} \\]_{u}^{1} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\]_{u}^{1} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\]_{u}^{1} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(\mathrm{Sia})_{j}^{-}(R)_{y} \\ \mathbf{A} \bullet \end{bmatrix}_{t}^{1} \\ \mathbf{A} \bullet \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{b}^{-}(\mathrm{Sia})_{j}^{-}(\mathrm{Sia})_{$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 40P

```
CHO, BHK, 293 cells, Vero expressed TNK tPA
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
ST3Gal3
3. CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 40Q

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 40R

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; \mathbf{v} - \mathbf{y} = 0.

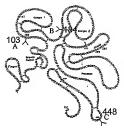
1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 40S

WO 2004/099231 PCT/US2004/011494

169/497



$$A \stackrel{\text{(Fuc)}_{i}}{\underset{\mid}{\text{GlcNAc-Gal)}_{a}l_{e^{-}}(\text{Sia)}_{i^{-}}(R)_{v}}}{\underset{\mid}{\text{Man}}} \underbrace{\begin{bmatrix} [\text{GlcNAc-(Gal)}_{a}l_{e^{-}}(\text{Sia)}_{i^{-}}(R)_{v} \\ [\text{GlcNAc-Gal)}_{a}l_{e^{-}}(\text{Sia})_{e^{-}}(R)_{w} \end{bmatrix}_{i}^{i}}_{\underset{\mid}{\text{Man}}} \underbrace{\begin{bmatrix} [\text{GlcNAc-(Gal)}_{a}l_{e^{-}}(\text{Sia})_{e^{-}}(R)_{w} \\ [\text{GlcNAc-(Gal)}_{a}l_{e^{-}}(\text{Sia})_{e^{-}}(R)_{y} \end{bmatrix}_{i}^{i}}_{\underset{\mid}{\text{glcNAc-(Gal)}_{a}l_{e^{-}}(\text{Sia})_{e^{-}}(R)_{y} \end{bmatrix}_{i}}^{\text{(R)}_{y}}$$

a-d, i, n-y (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

R" = glycosyl residue.

FIG. 40T

```
\label{eq:linear_transform} \begin{split} & \text{Insect cell expressed TNK tPA} \\ & \text{a-d, f, h, j-m, s, u, v-y} = 0; \\ & \text{e, g, i, q, r, t (independently selected)} = 0 \text{ or } 1. \end{split}
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 40U

```
Yeast expressed TNK tPA a-m=0; q-y (independently selected) = 0 to 1; p=1; R (branched or linear) = Man, oligomannose.
```

```
    Endoglycanase
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-PEG.
```

FIG. 40V

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

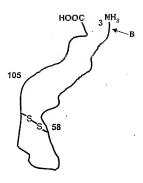
 CMP-SA-linker-Gal-UDP, ST3Gal3
 Galactosyltransferase, anti-TNF IG chimera produced in CHO.

a-m, r-u (independently selected) = 0 or 1; p, q = 1; n = 0; v-y (independently selected) = 0 or 1; R = Iinker-anti-TNF IG chimera protein.

FIG. 40W

WO 2004/099231 PCT/US2004/011494

172/497



a-c, e (independently selected) = 0 or 1; d=0; R= modifying group, mannose, oligomannose.

FIG. 41A

WO 2004/099231 PCT/US2004/011494

173/497

```
CHO, BHK, 293 cells, Vero expressed IL-2
a-c, e (independently selected) = 0 or 1; d = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1
a-d, e (independently selected) = 0 or 1;
```

FIG. 41B

R = PEG.

```
Insect cell expressed IL-2
a, e (independently selected) = 0 or 1;
b, e, d = 0.

1. Galactosyltransferase, UDP-Gal
```

- Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal1
- a, c, d, e (independently selected) = 0 or 1; R = PEG.

FIG. 41C

```
E. coli expressed IL-2
a-e = 0.

1. GaiNAc Transferase, UDP-GaiNAc
2. CMP-SA-PEG, siatyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0: R = PEG.
```

FIG. 41D

```
NSO expressed IL-2
a, e (independently selected) = 0 or 1;
b, c, d = 0

1. CMP-SA-levulinate, ST3Gal1
2. H_4N_2-PEG

a, c, d, e (independently selected) = 0 or 1;
b = 0; R = PEG.
```

FIG. 41E

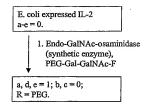


FIG. 41F

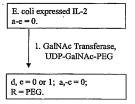


FIG. 41G

for some N-linked structures of A.

176/497

2 peptides

A and A' - N-linked sites

B - O-linked sites

$$(\operatorname{Fuc})_{i} = (\operatorname{Gal})_{a} \cdot (\operatorname{Sia})_{j} \cdot (\operatorname{Sia})_{j} \cdot (\operatorname{R})_{v} \cdot (\operatorname{R})_{v} \cdot (\operatorname{Gal})_{a} \cdot (\operatorname{Sia})_{j} \cdot (\operatorname{R})_{v} \cdot (\operatorname{R})_{v} \cdot (\operatorname{Gal})_{a} \cdot (\operatorname{Sia})_{a} \cdot (\operatorname{R})_{v} \cdot (\operatorname{R})_{v} \cdot (\operatorname{Gal})_{a} \cdot ($$

A'←-GlcNAc-GlcNAc

(R')

a-d, i, n-u (independently selected) = 0 or 1. aa, bb (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z=0; R = polymer, glycoconjugate.

FIG. 42A

```
CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1; v-z = 0.
```

1. Sialidase
 ▼ 2. CMP-SA-PEG, ST3Gal3

```
e-h = 1 to 4;
aa, bb, a-d, i, n, q-u (independently selected) = 0 or 1;
o, p, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 42B

```
CHO, BHK, 293S cells, Vero, MDCK, 293S, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1; v-z = 0.
```

```
1. Sialidase
2. CMP-SA-PEG, ST3Gal3
3. ST3Gal1, CMP-SA
```

```
e-h = 1 to 4;
aa, bb, a-d, i, n, p-u (independently selected) = 0 or 1;
o, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 42C

WO 2004/099231 PCT/US2004/011494

178/497

```
CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed Factor VIII. e-h= 1 to 4; aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1; v-z = 0.
```

1. CMP-SA-PEG, ST3Gal3

```
e-h = 1 to 4;
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;
z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 42D

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected) 0 or 1;
v-z = 0.
```

1. CMP-SA-PEG, ST3Gal1

```
e-h = 1 to 4;
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;
z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 42E

CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1; v-z = 0.

1. CMP-SA-PEG, α2,8-ST

e-h = 1 to 4; aa, bb, a-d, i, n-y (independently selected) = 0 or 1; z = 0; j-m (independently selected) = 0 to 2; v-y (independently selected) = 1, when j-m (independently selected) is 2; R = PEG.

FIG. 42F

2 peptides
A OF A - N-linked sites
B - O-linked sites

$$A \leftarrow \begin{array}{c} (\operatorname{Fuc})_{i} \\ -\operatorname{GlcNAc} -\operatorname{GlcNAc-Man} \\ (R')_{dd} \end{array} \qquad \begin{array}{c} (\operatorname{GlcNAc-(Gal)}_{a}|_{a}^{-}(\operatorname{Sia})_{j}^{-}(R)_{v})_{i} \\ (\operatorname{GlcNAc-(Gal)}_{b}|_{i}^{-}(\operatorname{Sia})_{k}^{-}(R)_{w})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{d}|_{g}^{-}(\operatorname{Sia})_{i}^{-}(R)_{x})_{i} \\ (\operatorname{GlcNAc-(Gal)}_{d}|_{g}^{-}(\operatorname{Sia})_{m}^{-}(R)_{y})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{Sia})_{m}^{-}(R)_{y})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{Sia})_{m}^{-}(R)_{g})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{Sia})_{m}^{-}(R)_{g})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{Sia})_{m}^{-}(R)_{g})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{Sia})_{m}^{-}(\operatorname{Sia})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{Sia})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{-}(\operatorname{GlcNAc-(Gal)}_{m})_{g} \\ (\operatorname{GlcNAc-(Gal)}_{m}|_{g}^{$$

a-d, i, n-u, (independently selected) = 0 or 1. aa, bb, cc, dd (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z = 0; R = modifying group, mannose, oligo-mannose.

R = modifying group, mannose, oligo-mannose R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 42G

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1; dd, v-z = 0.
```

1. CMP-SA-levulinate, ST3Gal3, 2. H₄N₂-PEG

```
e-h = 1 to 4;
aa, bb, cc, a-d, i, n-u (independently selected) = 0 or 1;
dd, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 42H

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; as, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1; dd, v-z=0.
```

```
1. endo-H
2. galactosyltransferase, UDP-Gal-PEG
```

```
e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z=0; R' = -Gal-PEG.
```

FIG. 421

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

```
    ST3Gal3, CMP-SA
    2. endo-H
    3. galactosyltransferase, UDP-Gal-PEG
```

e-h = 1 to 4; aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1; cc, v-z = 0; R' = -Gal-PEG.

FIG. 42J

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

```
1. mannosidases
2. GNT 1 & 2, UDP-GlcNAc
```

3. galactosyltransferase, UDP-Gal-PEG

```
e-h=1 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z=0; R=PEG.
```

FIG. 42K

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1; dd, v-z = 0.
```

- mannosidases
- 2. GNT-1,2, 4 & 5; UDP-GlcNAc
- ↓ 3. galactosyltransferase, UDP-Gal
 4. ST3Gal3, CMP-SA

```
e-h = 1 to 4;
aa, bb, cc, a-d, i, j-q (independently selected) = 0 or 1;
dd, v-z=0.
```

FIG. 42L

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1; dd, v-z = 0.
```

```
    1. mannosidases
    2. GNT-1, ÚDP-GlcNAc-PEG
```

```
e-h = 0 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0.
```

FIG. 42M



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \text{(Fuc)}_{i} \\ \text{(GlcNAc-(Gal),]}_{a}^{-} (\text{Sia)}_{i}^{-} (\text{R})_{v} \\ \text{(GlcNAc-GlcNAc-Man)} \\ \text{(GlcNAc-(Gal),]}_{a}^{-} (\text{Sia)}_{i}^{-} (\text{R})_{v} \\ \text{(Sia)}_{i}^{-} (\text{R})_{v} \\ \text{(GlcNAc-(Gal),]}_{a}^{-} (\text{Sia)}_{i}^{-} (\text{R})_{v} \\ \text{(GlcNAc-(Gal),]}_{a}^{-} (\text{Sia)}_{m}^{-} (\text{R})_{v} \\ \text{(GlcNAc-(Gal),]}_{a}^{-} (\text{Sia})_{m}^{-} (\text{Sia})_$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 43A

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.
```

 Sialidase
 CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 43B

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),
ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 43C

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 43D

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>a</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 43E

```
CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1;
j-m (independently selected) = 0-20;
```

v-y (independently selected) = 0.

FIG. 43F

WO 2004/099231 PCT/US2004/011494

188/497



$$A \leftarrow \left(\begin{array}{c} \text{Fuc} j_i \\ \text{GlcNAc-Gal} j_{a_i} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-Gal} j_{a_i} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-Man} \\ \text{Man} \left(\begin{array}{c} \text{[GlcNAc-(Gal)_a]_e} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a]_e} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a]_e} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a]_e} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a]_e} \text{-} \left(\text{Sia} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \text{-} \left(\text{Ri} j_i \right)_s \right) \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \right)_s \\ \text{GlcNAc-(Gal)_a} \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \right)_s \\ \text{GlcNAc-(Gal)_a} \\ \text{GlcNAc-(Gal)_a} \text{-} \left(\text{Ri} j_i \right)_s \\ \text{GlcNAc-(Gal)_a} \\ \text{$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 43G

```
Insect cell expressed Urokinase.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-n = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 43H

```
Yeast expressed Urokinase.

a-n=0;

q-y (independently selected) = 0 to 1;

p=1; R (branched or linear) = Man, oligomannose.
```

```
    Endoglycanase
    Galactosyltransferase, UDP-Gal
    CMP-SA-PEG, ST3Gal3
```

```
a-m, p-y = 0; n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 431

```
CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.
```

- CMP-SA-linker-SA-CMP, ST3Gal3
- ST3Gal1, desialylated Urokinase produced in CHO.
- ↓ 3. CMP-SA, ST3Gal3, ST3Gal1

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-Urokinase.
```

/ FIG. 43.1

```
Isolated Urokinase.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0; n = 0;

Sia (independently selected) = Sia or SO<sub>4</sub>;

Gal (independently selected) = Gal or GalNAc;

GicNAc (independently selected) = GicNAc or GicNAc-Fuc.
```

sulfohydrolase
 CMP-SA-PEG, sialyltransferase

```
a-d, i-m, q-u (independently selected) = 0 or 1;
n = 0; e-h = 1; Sia = Sia;
Gal (independently selected) = Gal or GalNAc;
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.
v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 43K

Isolated Urokinase.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; n = 0; v-y = 0;

Sia (independently selected) = Sia or SO₄;

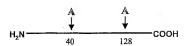
Gal (independently selected) = Gal or GalNAc;

GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.

sulfohydrolase, hexosaminidase
 UDP-Gal-PEG, galactosyltransferase

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-n = 0; Gal (independently selected) = Gal; GleNAc (independently selected) = GleNAc or GleNAc-Fuc; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 43L



$$A = \begin{array}{c} \text{(Fuc)}_{i_{j}} \\ \text{GlcNAc-GlcNAc-Man} \\ \text{Man} \\ \text{[[GlcNAc-(Gal)_{g}]_{g^{-}}(Sia)_{j} - (R)_{w})}_{t_{j}} \\ \text{[[GlcNAc-(Gal)_{g}]_{g^{-}}(Sia)_{l} - (R)_{w})}_{t_{j}} \\ \text{[[GlcNAc-(Gal)_{g}]_{g^{-}}(Sia)_{m^{-}}(R)_{y})}_{t_{j}} \\ \text{[[GlcNAc-(Gal)_{g}]_{g^{-}}(Sia)_{m^{-}}(R)_{y}}_{t_{j}} \\ \text{[[GlcNAc-(Gal)_{g}]_{g^{-}}(Sia)_{m^{-}}(R)_{y}}_{t_{j}} \\ \text{[[GlcNAc-(Gal)_{g}]_{g^{-}}(R)_{y}}_{t_{j}} \\ \text{[[GlcNAc-(Ga$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 44A

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- Sialidase
- CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 44B

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 44C

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. Sialidase
2. CMP-SA-PEG (16 mol eq), ST3Gal3
3. CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 44D

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. CMP-SA-levulinate, ST3Gal3, buffer, salt

▼ 2. H₄N}-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

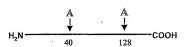
FIG. 44E

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. CMP-SA, α2,8-ST
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1;
j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

FIG. 44F



$$A^{\bullet} \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}_{a}\right]_{e}^{-} \left(\operatorname{Sia}\right)_{j}^{-} \left(\operatorname{R}\right)_{v} \right]_{r}^{}}_{\left[\left[\operatorname{GloNAc-(Gal)_{a}}\right]_{e}^{-} \left(\operatorname{Sia}\right)_{k}^{-} \left(\operatorname{R}\right)_{w} \right]_{q}^{}} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{r}^{-} \left(\operatorname{Sia}\right)_{r}^{-} \left(\operatorname{R}\right)_{w} \right]_{q}^{}}_{\left[\left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{y} \right]_{q}^{}}_{q} \\ \underbrace{ \begin{bmatrix} \left[\operatorname{GloNAc-(Gal)_{a}}\right]_{h}^{-} \left(\operatorname{Sia}\right)_{h}^{-} \left(\operatorname{R}\right)_{h}^{-} \left(\operatorname{R}\right)_{h}^$$

a-d, i, n, p-n (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 44G

```
Insect cell expressed DNase I. a-d, f, h, j-n, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1; j-n = 0; v-y (independently selected) = 1, when e-h (independently selected) is 1; R = PEG.
```

FIG. 44H

```
Yeast expressed DNase I.
a-n = 0;
q-y (independently selected) = 0 to 1;
p = 1; R (branched or linear) = Man, oligomannose.
```

```
    Endoglycanase
    Galactosyltransferase, UDP-Gal
    CMP-SA-PEG, ST3Gal3
```

```
a-n, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

FIG. 441

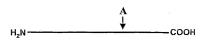
WO 2004/099231 PCT/US2004/011494

198/497

CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- CMP-SA-linker-SA-CMP, ST3Gal3
 ST3Gal1, desialylated alpha-1 Proteinase inhibitor.
- 3. CMP-SA, ST3Gal3, ST3Gal1
- a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0;
- v-y (independently selected) = 0 or 1;
- R = linker- alpha-1-Proteinase inhibitor.

FIG. 44J



$$(Fuc)_{i} \\ A \leftarrow GlcNAc\text{-}GlcNAc\text{-}Man \\ (R')_{n} \\ (RC)_{n} \\ (RGlcNAc\text{-}Gal)_{a}l_{r} - (Sia)_{s} - (R)_{w} \\ (RGlcNAc\text{-}Gal)_{a}l_{r} - (Sia)_{s} - (R)_{w} \\ (RGlcNAc\text{-}Gal)_{a}l_{r} - (Sia)_{r} - (R)_{s} \\ (RGlcNAc\text{-}Gal)_{a}l_{r} - (Sia)_{m} - (R)_{y} \\ (RGlcNAc\text{-}Gal)_{a}l_{r} - (RGlCNAc\text{-}Gal)_{$$

a-d, i, r-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
'
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 45A

```
CHO, BHK, 293 cells, Vero expressed Insulin.
a-m, r-u (independently selected) = 0 or 1;
n = 0; v-y = 0; z = 1.
```

```
    Sialidase
    CMP-SA-PEG, ST3Gal3
```

```
a-m; r-u (independently selected) = 0 or 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
n = 0; R = PEG; z = 1.
```

FIG. 45B

```
Insect cell expressed Insulin.

a-h, j-n, s-y = 0;

i, r (independently selected) = 0 or 1; z = 1.

1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
when e, g (independently selected) is 1;
z = 1; R = PEG.
```

FIG. 45C

Yeast expressed Insulin.

a-n=0; r-y (independently selected) = 0 to 1;

z = 1;

R (branched or linear) = Man, oligomannose or polysaccharide.

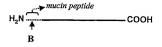
1. Endo-H

2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z= 0; n = 1; R' = -Gal-PEG.

FIG. 45D





a-c, e (independently selected) = 0 or 1; d = 0; R = polymer

FIG. 45E

CHO, BHK, 293 cells, Vero expressed insulinmucin fusion protein.

a-c, e (independently selected) = 0 or 1; d = 0

Sialidase
 CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 45F

Insect cell expressed Insulin-mucin fusion protein. a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

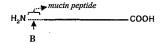
FIG. 45G

E. coli expressed Insulin-mucin fusion protein. a-e=0.

- GalNAc Transferase, UDP-GalNAc
 CMP-SA-PEG, sialyltransferase
- c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 45H





$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_b \\ -\operatorname{GalNAc-(Gal)_a-(Sia)_c-(R)_d} \end{bmatrix}_c$$

$$C \leftarrow (R')_n$$

a-c, e (independently selected) = 0 or 1; d=0; R= modifying group, mannose, oligo-mannose.

FIG. 451

WO 2004/099231 PCT/US2004/011494

206/497

E. coli expressed Insulin-mucin fusion protein. a-e, n=0.

GalNAc Transferase,
 UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

FIG. 45J

E. coli expressed Insulin-mucin fusion protein. a-e, n=0.

- GalNAc Transferase,
 UDP-GalNAc-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

FIG. 45K

WO 2004/099231 PCT/US2004/011494

207/497

E. coli expressed Insulin (N)—no mucin peptide. a-e, n=0.

- 1. NHS-CO-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 45L

$$(Acyl)_{0-1}HN \xrightarrow{4} \xrightarrow{4} \xrightarrow{146} COOH$$

$$A \qquad A$$

$$A \leftarrow \begin{bmatrix} (\operatorname{Fuc})_i & & & & & \\ (\operatorname{Fuc})_i & & & \\ (\operatorname{Fuc})_i & & & & \\ (\operatorname{Fuc})_i & & & \\ (\operatorname{Fuc$$

a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 46A

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

1. Sialidase

 CMP-SA-linker-lipid-A, ST3Gal3

a-d, i-m, q-u, aa (independently selected) = 0 or 1; o, p, z = 0; n, e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = linker-lipid-A.

FIG. 46B

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

1. sialidase

2. CMP-SA-linker-tetanus toxin, ST3Gal1

3. CMP-SA, ST3Gal3

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1; o, v-y = 0; n, e-h = 1; R = tetanus toxin.

FIG. 46C

```
NSO expressed M-antigen.
a-d, i-n, o-u, aa (independently selected) = 0 or 1;
e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.
```

```
    α-galactosidase
    CMP-SA, ST3Gal3
    CMP-SA-KLH, ST3Gal1
```

```
a-d, i-n, p-u, z, as (independently selected) = 0 or 1;
e-h = 1; o, v-y = 0;
z = 1, when p = 1;
R = KLH.
```

FIG. 46D

```
Yeast expressed M-antigen.
a-p, z = 0; q-y, aa (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

```
1. α1,2-mannosidase
2. GNT 1,
UDP-GlcNAc-linker-diphtheria toxin.
```

```
e, q, l, m, r, t, u, v, aa (independently selected) =0 or l; a-d, f-h, j, k, n-p, s, w-z = 0; Sia = Man; R = linker-diphtheria toxin.
```

FIG. 46E

WO 2004/099231 PCT/US2004/011494

211/497

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

1. CMP-SA-levulinate, ST3Gal3, 2. H₄N₂-linker-DNA

a-d, i-m, o-y, as (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = linker-DNA.

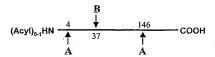
FIG. 46F

CHO, BHK, 293 cells, Vero expressed M-antigen.
a-d, i-n, o-u, aa (independently selected) = 0 or 1;
e-h = 1; v-z = 0.

1. CMP-SA, poly- α 2,8-ST

a-d, i, n-u, aa (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-100; v-z (independently selected) = 0.

FIG. 46G



$$\begin{array}{c} A & \longleftarrow \begin{pmatrix} (\operatorname{Fuc})_i & & & \\ (\operatorname{Fuc})_i & & & \\ (\operatorname{GlcNAc-Man})_{\operatorname{ClcNAc-Man}} & & & \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}_{\operatorname{al}}^{-}}(\operatorname{Sia})_{\operatorname{j}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{g}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{cl}_{\operatorname{al}}^{-}}(\operatorname{Sia})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{l}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}_{\operatorname{l}^{-}}}(\operatorname{Sia})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{l}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}^{-}}(\operatorname{Sia})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{l}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}^{-}}(\operatorname{Sia})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{l}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}^{-}}(\operatorname{Sia})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{l}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}^{-}}(\operatorname{Sia})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{l}^{-}}(\operatorname{R})_{\operatorname{w}})_{\operatorname{l}} \\ (\operatorname{GlcNAc-(Gal)}_{\operatorname{al}^{-}}(\operatorname{R})_{\operatorname{l}^{-}}(\operatorname{R})$$

a-d, i, n, q-u, aa, bb, (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-p (independently selected) = 0 to 100. Cc, v-y = 0; R = modifying group, mannose, oligo-mannose. R'=H, glycosyl residue, modifying group, glycoconjugate.

FIG. 46H

WO 2004/099231 PCT/US2004/011494

213/497

```
Insect cell expressed M-antigen. a-d, f, h, j-m, o, p, s, u, v-z, cc = 0; bb = 1; e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

l. GNT-2, UDP-GlcNAc-linker-Neisseria protein

```
a, c, e, g, i, n, q, r, t, v, x, aa (independently selected) = 0 or 1; b, d, f, h, j-p, s, u, w, y, z, cc = 0; bb = 1; R = -linker-Neisseria protein.
```

FIG. 46!

```
Yeast expressed M-antigen.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

```
    1. Endoglycanase
    2. Galactosyltransferase,
    UDP-Gal-linker-Neisseria protein
```

```
a-p, r-z, bb = 0;
q, aa, cc (independently selected) = 0 or 1;
R' = -Gal-linker-Neisseria protein.
```

FIG. 46J

Yeast expressed M-antigen.

a-p, z, cc = 0;

q-y, as (independently selected) = 0 to 1; bb = 1;

R (branched or linear) = Man, oligomannose;

GalNAc = Man.

- 1. mannosidases
- 2. GNT 1 & 2, UDP-GlcNAc
 3. UDP-Gal, Galactosyltransferase,
 4. CMP-SA, sialyltransferase

a, c, e, g, j, l, q, r, t, aa (independently selected) = 0 or 1; b, d, f, h, k, m-p, s, u-z, cc = 0; bb = 1.

FIG. 46K



$$(Fuc)_{i} \\ A \leftarrow GlcNAc \\ -GlcNAc \\$$

a-d, i, r-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1;
R - modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 47A

WO 2004/099231 PCT/US2004/011494

216/497

CHO, BHK, 293 cells, Vero expressed Growth Hormone.

a-m, r-u (independently selected) = 0 or 1; n = 0; v-y = 0; z = 1.

Sialidase
 CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; n = 0; R = PEG; z = 1.

FIG. 47B

$$\label{eq:continuous} \begin{split} & \text{Insect cell expressed growth hormone.} \\ & \text{a-h, j-n, s-y} = 0; \\ & \text{i, r (independently selected)} = 0 \text{ or l; } z = 1. \end{split}$$

1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0; e, g, i, r, t, v, x (independently selected)= 0 or 1; v, x (independently selected) = 1, when e, g (independently selected) is 1; z = 1; R = PEG.

FIG. 47C

WO 2004/099231 PCT/US2004/011494

217/497

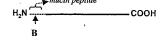
Yeast expressed growth hormone. a-n = 0; r-y (independently selected) = 0 to 1; z = 1; R (branched or linear) = Man, oligomannose or polysaccharide.

> l. Endo-H 2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z= 0; n = 1; R' = -Gal-PEG.

FIG. 47D





$$\mathbf{B} \leftarrow \begin{pmatrix} (\operatorname{Sia})_b \\ -\operatorname{GalNAc-(Gal)}_a - (\operatorname{Sia})_c - (\mathbf{R})_d \end{pmatrix}$$

a-c, e (independently selected) = 0 or 1; d = 0:

R = modifying group, mannose, oligomannose.

FIG. 47E

CHO, BHK, 293 cells, Vero expressed growth hormone-mucin fusion protein.

a-c, e (independently selected) = 0 or 1; d = 0

- Sialidase
- 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 47F

Insect cell expressed Growth Hormone-mucin fusion protein.

a, e (independently selected) = 0 or 1; b, c, d = 0.

Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

FIG. 47G

E. coli expressed growth hormone-mucin fusion protein.

a-e = 0.

GalNAc Transferase, UDP-GalNAc
 CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 47H

E. coli expressed growth hormone-mucin fusion protein. a-e, n = 0.

GalNAc Transferase,
 UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

FIG. 471

E. coli expressed growth hormone-mucin fusion protein.

a-e, n=0.

1. GalNAc Transferase,
UDP-GalNAc-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA. ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

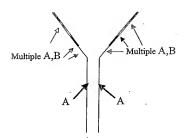
FIG. 47J

E. coli expressed growth hormone
(N)—no mucin peptide.
a-e, n = 0.

NHS-CO-linker-SA-CMP
 ST3Gal3, asialo-transferrin
 CMP-SA, ST3Gal3

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 47K



a-d, i-m, q-u, w, z, nn, ww, zz (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4.

n, v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 48A

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion. a-m, o-u, as (independently selected) = 0 or 1; n = 1; $v \cdot z = 0$.

```
    CMP-SA, ST3Gal1
    galactosyltransferase, UPD-Gal
    CMP-SA-PEG, ST3Gal3
```

```
a-m, o-u, v-y, aa (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.
```

FIG. 48B

```
CHO, BHK, 293 cells, Vero expressed
TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.
```

```
1. sialidase

✓ 2. CMP-SA-PEG, ST3Gal1
```

```
a-i, p-u, z, aa (independently selected) = 0 or 1;

n = 1; o, j-m, v-y = 0; R = PEG.
```

FIG. 48C

```
CHO, BHK, 293 cells, Vero expressed
TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.
```

1. galactosyltransferase, UPD-Gal-PEG

```
a-m, o-u, v-y, as (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.
```

FIG. 48D

```
CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion. a-m, o-u, as (independently selected) = 0 or 1; n = 1; v-z = 0.
```

```
1. CMP-SA, ST3Gall
2. galactosyltransferase, UPD-Gal-PEG
```

```
a-m, o-u, v-y, aa (independently selected) = 0 or 1;

n = 1; z = 0; R = PEG.
```

FIG. 48E

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion. a-m, o-u, as (independently selected) = 0 or 1; n=1; v-z=0.

1. CMP-SA-levulinate, ST3Gal1

▼ 2. H₄N₂-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1; n=1; z=0; R=PEG.

FIG. 48F

CHO, BHK, 293 cells, Vero expressed TNF Receptor IgG Fusion. a-m, o-u, as (independently selected) = 0 or 1; n = 1; v-z = 0.

1. CMP-SA-PEG, α 2,8-ST

a-i, o, q-u, v-z, as (independently selected) = 0 or 1; n = 1; j-m, p (independently selected) = 0 to 2; v-z (independently selected) = 1, when j-m, p (independently selected) is 2; R = PEG.

FIG. 48G